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ACUTE ORAL TOXICITY AND ANTI TUSSIVE EFFECT OF MUKOSAN SYRUP (POLY HERBAL FORMULATION) ON SO_2 INDUCED COUGH MODEL.

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ABSTRACT

Introduction: The various serious conditions of respiratory system like pneumonia, asthma, pulmonary HT and TB have one common cardinal symptom i.e. Coughing. The currently available various allopathic drugs like anti-tussive, expectorants, mucolytics etc., are being used for the treatment of cough which gives symptomatic relief in the most extents. But the involvement of debilitating side effects is major drawback of these drugs. Therefore, it is a unmet need to develop safe and effective antitussive therapeutic option for treatment of persistent cough as alternative to existing medicine. **Aim:** To evaluate Mukosan Syrup (poly herbal formulation) for acute oral toxicity in mice and antitussive effect in So2 induced cough model**. Method:** The present study was certified by IAEC (SKPCPER/IAEC/2016-02/02) as per the CPCSEA. The OECD guideline AOT-425 was followed during assessment of acute oral toxicity of test drug to know single dose toxicity. The selected animals were subjected to randomization with irrespective of their gender. A limit single dose (2000 mg/kg) of test drug was given to each mouse at 48 h intervals and observed periodically for 14 days for any clinical sign of toxicity or mortality. The modified and simplified method describe by Miyagoshi et al., 1986 was adopted for evaluation of antitussive effect in SO2 induced cough model in mice. **Results:** There were neither any physical - behavioral changes nor mortality observed in any animal during study period. The test drug showed significant decrease in cough bouts in compression to standard drug treated (codeine sulphate) group. **Conclusion:** The No-Observed-Adverse-Effect-Level (NOAEL) of Mukosan Syrup is 2000 mg/kg as it did not produce any toxicity at this dose. The significant decrease in cough bouts favors potential antitussive effect of Mukosan Syrup.

KEYWORDS: Anti tussive, Acute oral toxicity, Mukosan Syrup, Mortality, NOAEL.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and many of modern drugs have been isolated from natural sources, particularly from plants.[1] The concept of poly herbal formulation (PHF) is well documented in the ancient literature and they have better and expanded therapeutic potential as compared to the single herb.[2] The public interest in alternative system of therapies found increased in entire world during last decades.[3] Though, these PHFs are presumed as safe and effective[4] alternative medicines for treatment of various diseases, toxic potential of some herbal combinations is need to be tasted for provide adequate database regarding toxic properties of PHFs.[5]

The foreign particles or airway secretions can be expelled from the respiratory track through Coughing.[6] The drugs used to treat cough in present scenario are the most widely used as over-the-counter drugs world wide inspite of a recent analysis suggesting that there is a little evidence to such drugs produce any meaningful efficacy. The primary action of available cough cure medicines (opiates, dextromethorphan etc.) is on the central cough pathway. The significant side effects of these agents such as constipation, respiratory depression, dependence, drowsiness and death from this action limit their use in human and thus highly unsatisfactory.[7] Therefore, efforts have been trying to use of herbal formulations to treat the disease without any harmful effects. There are so many plants available to treat the airway infections like cold, cough, bronchial affections, pneumonia and expectoration. With this approach, numerous polyherbal formulations have been produced which help in the treatment of different types of cough.[8]

The present study has been conducted to test the acute oral toxicity of Mukosan Syrup (poly herbal formulation) to develop its NOAEL and also to establish its efficacy as potential anti tussive medicine.

AIMS AND OBJECTIVES

1. To evaluate acute oral toxicity of Mukosan Syrup on Swiss Albino Mice.
2. To evaluate antitussive effect of Mukosan Syrup in SO_2 induced cough in Mice.

MATERIALS AND METHODS

Test Material: The test drug (Mukosan Syrup) was prepared by maintaining all the GMP standards during manufacturing. The detail of Mukosan Syrup is mentioned below;

Table 1: Ingredients of Mukosan Syrup(Each 5 ml contains).

Sl. No.	Name of ingredient	Quantity
1	Ext. AdhatodaVasica	100mg
2	Ext. Glycyrrhiza glabra	100mg
3	Ext. Terminalia belerica	100mg
4	Ext. Ocimum sanctum	75mg
5	Ext. Solanum xanthocarpum	50mg
6	Ext. Zingiber officinale	25mg
7	Shuddha Tankan	25mg
8	Yavakshar	25mg
9	Mentha salvestris	3mg
10	Syrup Base	Q.S.
11	Colour Ponceau 4R	Q.S.

Method: The permission from IAEC (SKPCPER/IAEC/2016-02/02)as per the CPCSEA as well as from Ministry of Environment, Forest and Climate Change (MoFCC), Government of India was obtained before performed the study.

(A) Acute oral toxicity[9]: It was done by following OECD guideline AOT-425 to know single dose toxicity of test drug on swiss albino mice. All the Animals were kept in standard condition and feed with proper diet. They were acclimatized properly and divided in different groups prior to dosing. A limit single oral dose of 2000 mg/kg of test drug was given to each animal in sequence at 48 h intervals. The dosing detail is mentioned below;

Table 2: Individual animal dosing record.

Expt. Day	Animal No.	Gender	Volume dose (ml)
1st day	H	M	1
3rd day	B	M	1
5th day	T	M	1
7th day	HT	F	1
9th day	UM	F	1

Expt.: Experiment, H: Head, B: Body, T: Tail, HT: Head & Tail, UM: Unmarked, M: Male, F: Female

Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a total of 14 days for any clinical sign of toxicity or mortality. Body weight of all animals was also recorded once in a week.

(B) Anti-tussive effect[10]: This study was performed on So2 induced cough in mice to assess the anti-tussive effect of test drug. The detail of study is mentioned below;

The female Swiss albino mice having weighing range of 25-35g, age 8-12 weeks old, were kept in standard condition and acclimatized for seven days prior to dosing. They were randomized in to three groups with six animals in each group.

Table 3: Grouping of animals.

Group No.	Group name	Dose	No. of Animal
I	Disease control (DC)	NA	06
II	Standard drug treated (Std.) (codeine sulphate)	10mg/kg	06
III	Mukosan Syrup (MS)	400mg/kg	06

The antitussive activity against Sulphur dioxide (SO2) induced cough was evaluated by the method as describe by Miyagoshi et al., 1986[10] with modified and simplified. A Petri dish containing solution of 2 ml of 500 mg/ml of sodium hydrogen bisulfate (NaHSO3,S.D Fine-Chemical Ltd.) in double distilled water was placed in one compartment of chamber. After that, 0.2 ml of concentrated Sulphuric acid (H_2SO_4, S. D Fine-Chemical Ltd.) was added in to it by using a pipette to produce SO2 gas according to reaction as follow;

$$2NaHSO_3 + H_2SO_4 \longrightarrow 2SO_2 + Na_2SO_4 + 2H_2O$$

After 15 seconds, mice were placed in the other compartment of the chamber and exposed to SO_2 gas for 45 seconds. Then they were taken out from the chamber and put in to a big plate and cough bouts were counted for 5 minutes. In the same manner above procedure was repeated for all the mice of the treated groups and frequency of cough bouts was measured. The drugs were administered orally. The frequency of cough bouts was assessed before and after treatment.

Statistical Analysis

Arithmetic mean and standard error of mean were calculated from the individual observations. The data was expressed as mean ± S.E.M. Statistical difference between two groups is tested by using student's paired t-test. $p<0.05$ was considered as significant.

OBSERVATIONS AND RESULT

(A) Acute oral toxicity: All the animals were continuously observed for behavioural changes, autonomic profiles and other sign of toxicity or mortality up to a period of 14 days. The body weight, food intake and water intake were also observed on 1st, 7th and 14th day. There were no any physical and behavioural changes observed in any animal during observation period. Body weight of all animals did not reveal any significant change as compared to vehicle control group. Mortality was not found in any group.

Table 4: Individual animal weekly body weight record.

Animal No.	Sex	Experiment Day, Unit : gm			Mortality
		1st	7th	14th	
H	M	22	23	24	NIL
B	M	23	23	25	NIL
T	M	24	25	26	NIL
HT	F	28	29	29	NIL
UM	F	28	28	29	NIL

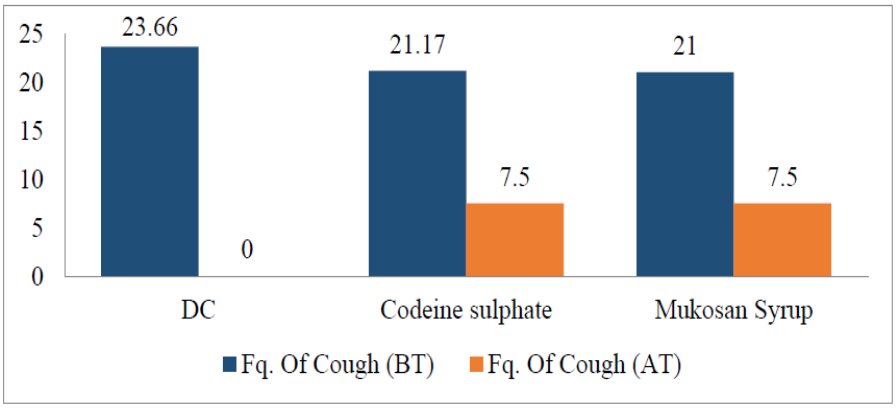
H: Head, B: Body, T: Tail, HT: Head & Tail, UM: Unmarked, M: Male, F: Female

(B) Anti-tussive effect

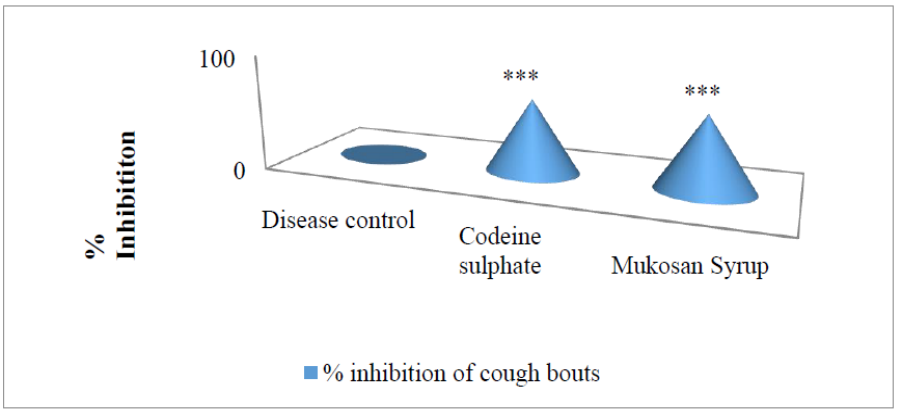
Table 5 Effect of Mukosan Syrup on frequency of cough bouts in SO2 induced cough model in mice (Value of cough bouts are expressed as mean ± S.E.M. (n=6).

Group No.	Treatment & Dose (mg/kg)	Frequency of cough bouts before treatment mean ± SEM	Frequency of cough bouts after treatment mean ± SEM	% inhibition of cough bouts
I	Disease control	23.66	-	-
II	Codeine sulphate(10 p.o)	21.17 ± 0.94	7.5 ± 0.67	64.55***
III	Mukosan Syrup(400p.o)	21.00 ± 1.5	7.5 ± 0.67	64.28***

***P<0.001 Vs Before treatment.



Graph 1: Frequency of cough bouts before & after treatment.



Graph 2 % Inhibition of cough bouts before & after treatment.

DISCUSSION

The acute toxicity profile and anti-tussive effect of Mukosan Syrup is not available till date. This study can consider as a pioneer step for the establishment of its safety profile.

The study was performed on Swiss Albino Mice for 14 days to evaluate any toxic effect of test drug at the single dose of 2000 mg/kg. Individual animal weekly body weight was recorded and found to be increasing during the observation period [**Table 4**]. Animal daily observation was recorded and did not reveal any physical or behavioral changes and mortality rate was Nil [**Table 4**]. This study reveals that Mukosan Syrup which is indicated in various respiratory conditions have no oral toxicity. Thus, it can be used safely for therapeutic purposes.

Mukosan Syrup is combination of various drugs which are indicated for respiratory dysfunction in ancient literature. *Glycyrrhiza glabra* used in management of cough and tuberculosis.[11] *Terminalia belerica*cures cures cough disorder and beneficial for throat.[12] *Adhatoda Vasica* have potential expectorant[13] and anti-tussive effect.[14] *Ocimum sanctum*[15], *Solanum xanthocarpum*[16] and *Zingiber officinale*[17] also have been established for their anti-tussive effect. *Shuddha Tankan* and *Yavakshar* have also potential expectorant effect. The test drug was assessed for anti-tussive activity in Sulphur dioxide (SO2) induced cough model which shows significant decrease in cough bouts and greater % inhabitation of cough bouts **[Table 5]** which suggests its potential anti tussive effect as compared to DC and Standard drug treated groups.

Therefore, Mukosan Syrup is safe and better effective in upper respiratory tract infections, common cold, cough, bronchitis, sinusitis, laryngitis, pharyngitis.

CONCLUSION

The No-Observed-Adverse-Effect-Level (NOAEL) of Mukosan Syrup is 2000 mg/kg as it does not have any toxic effect at this dose. The significant decrease in cough bouts favors potential anti tussive activity of test drug.

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